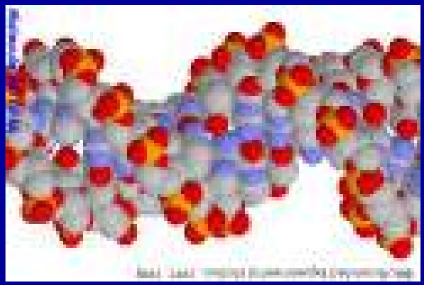
Recombinant DNA Technology



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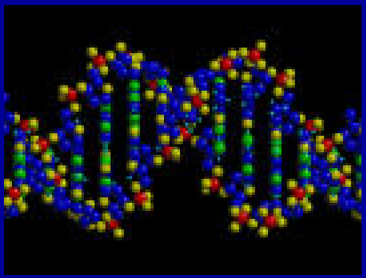
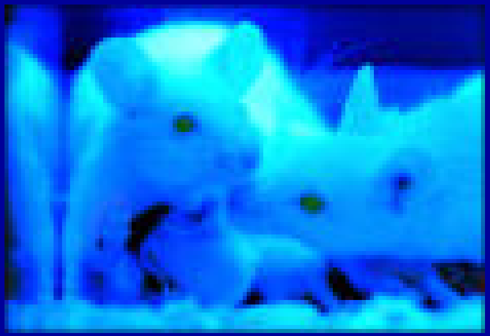


How to Mess with DNA

for Fun and Profit



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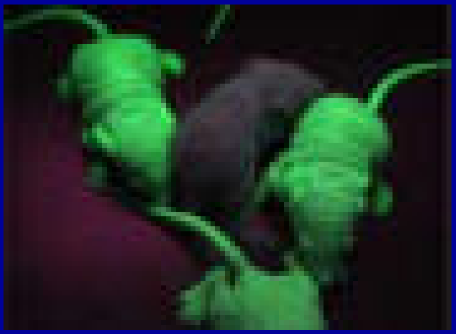
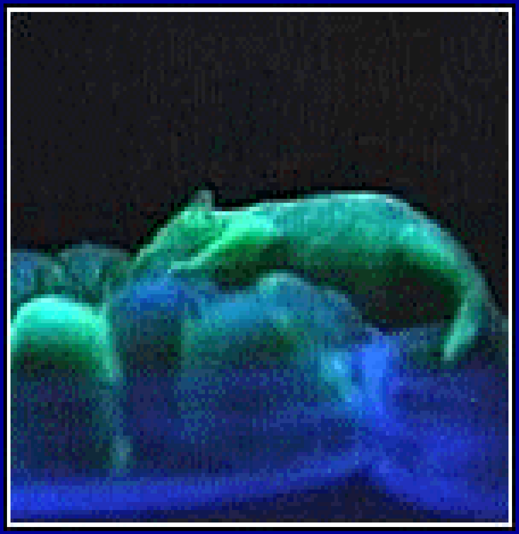


How to Make a Mouse Glow in the Dark



What the heck is Recombinant DNA?

Recombinant DNA is what you get when you combine DNA from two different sources.

For example:

Mouse + Human DNA

Human + Bacterial DNA

Viral + Bacterial DNA

Human + (other) Human DNA

(It's sort of like Frankenstein-DNA!)

Why Make Recombinant DNA?

Recombinant DNA Technology May Allow Us To:

- *Cure or treat disease*
- *Genetically modify our foods to increase flavor, yield, nutritional value or shelf-life*
- *Better understand human genetics*
- *Clone cells or organs*

Molecular Biology's Best Friends: *Bacteria*

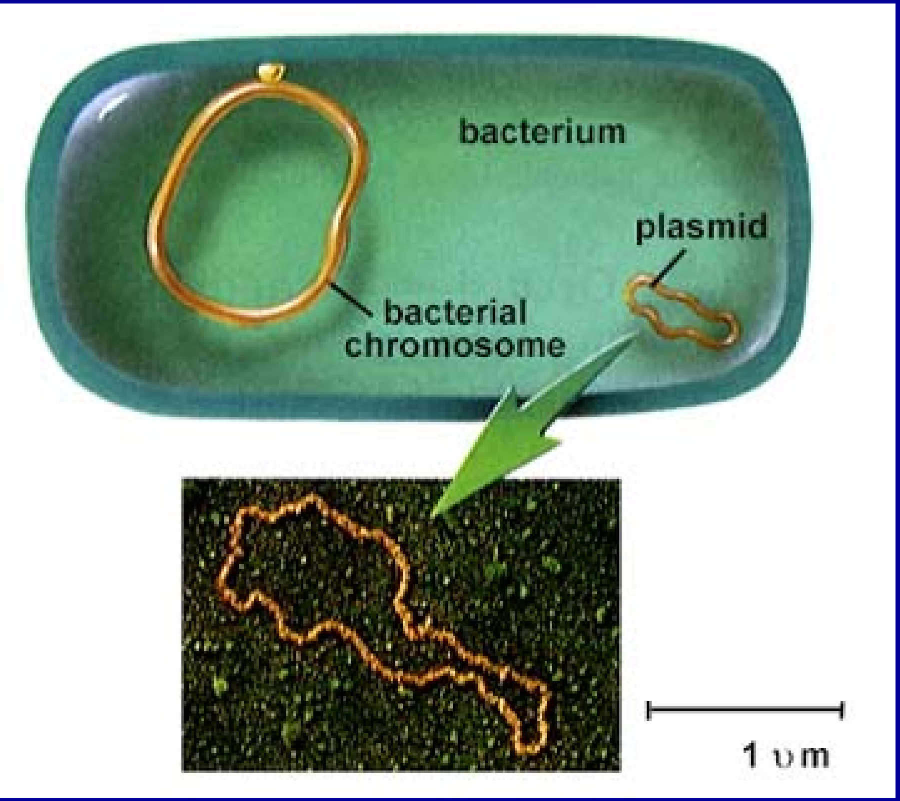
Why use bacteria?

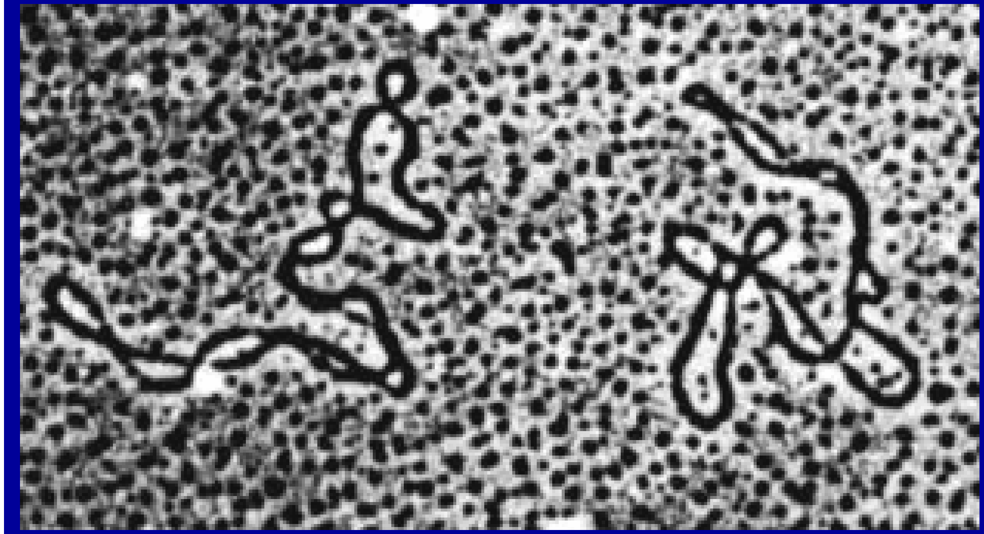
- They're relatively *simple* organisms.
- They reproduce *very quickly* and *asexually* (this means that the "daughter" cells will contain the exact same DNA as the "parent" cell).
- It's pretty easy to get DNA back into the bacteria after you've changed it.
- We can mess around with their DNA and kill a lot of them during our experiments and nobody gets mad. ;-)

Now for a little vocabulary…

Plasmids

Small, circular pieces of “extra” DNA found in bacteria.



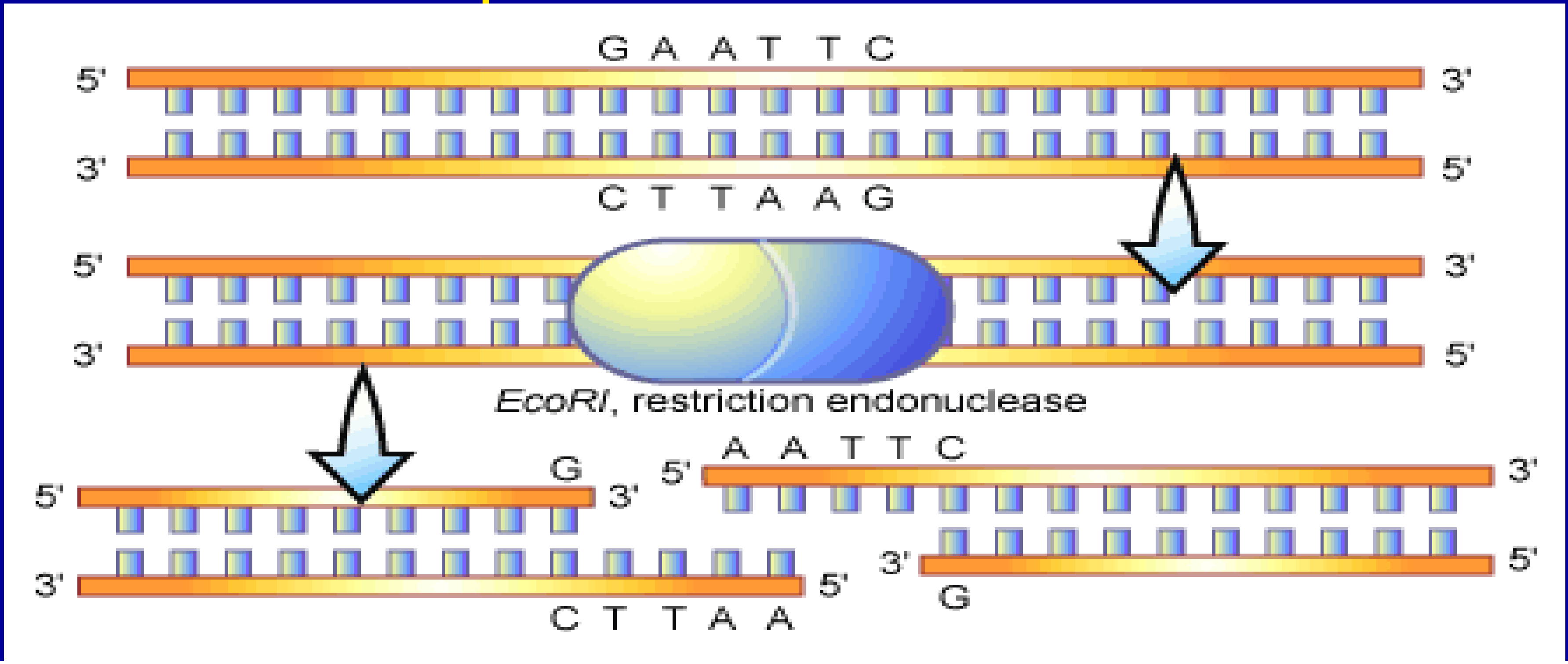


Plasmids often carry antibiotic resistance.

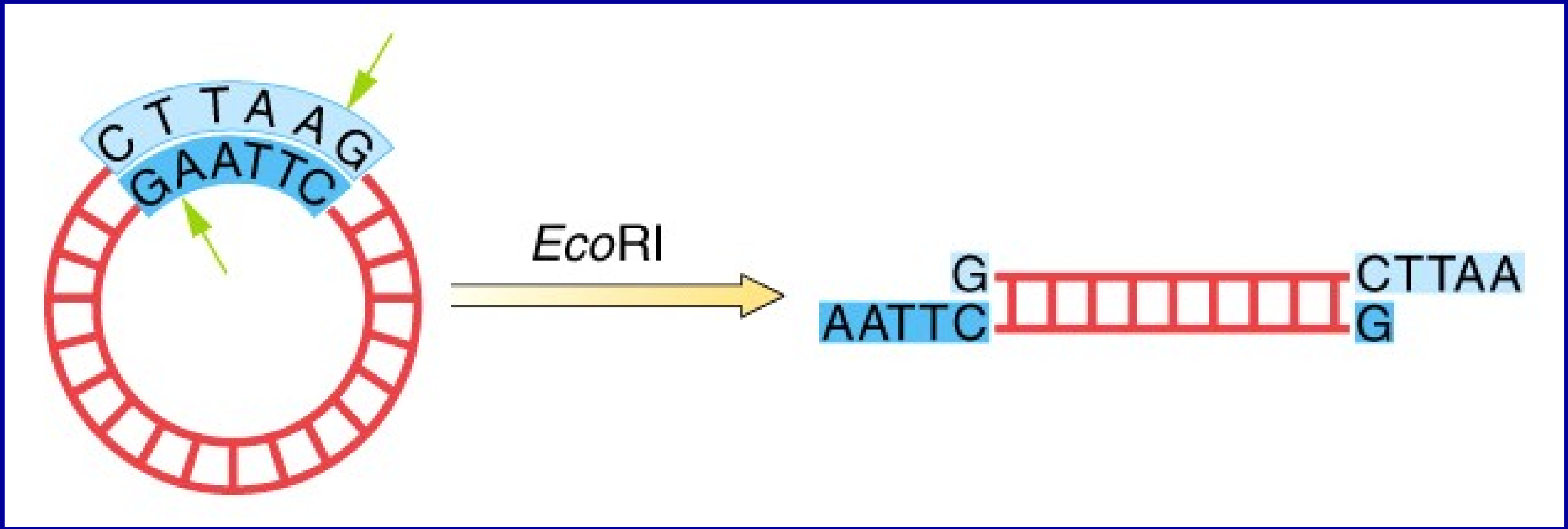
Restriction Enzymes: Molecular Scissors

A restriction enzyme (RE) is a specialized protein that cuts DNA in a *very specific* place.

- Different REs cut at different places along the nucleotide sequence.

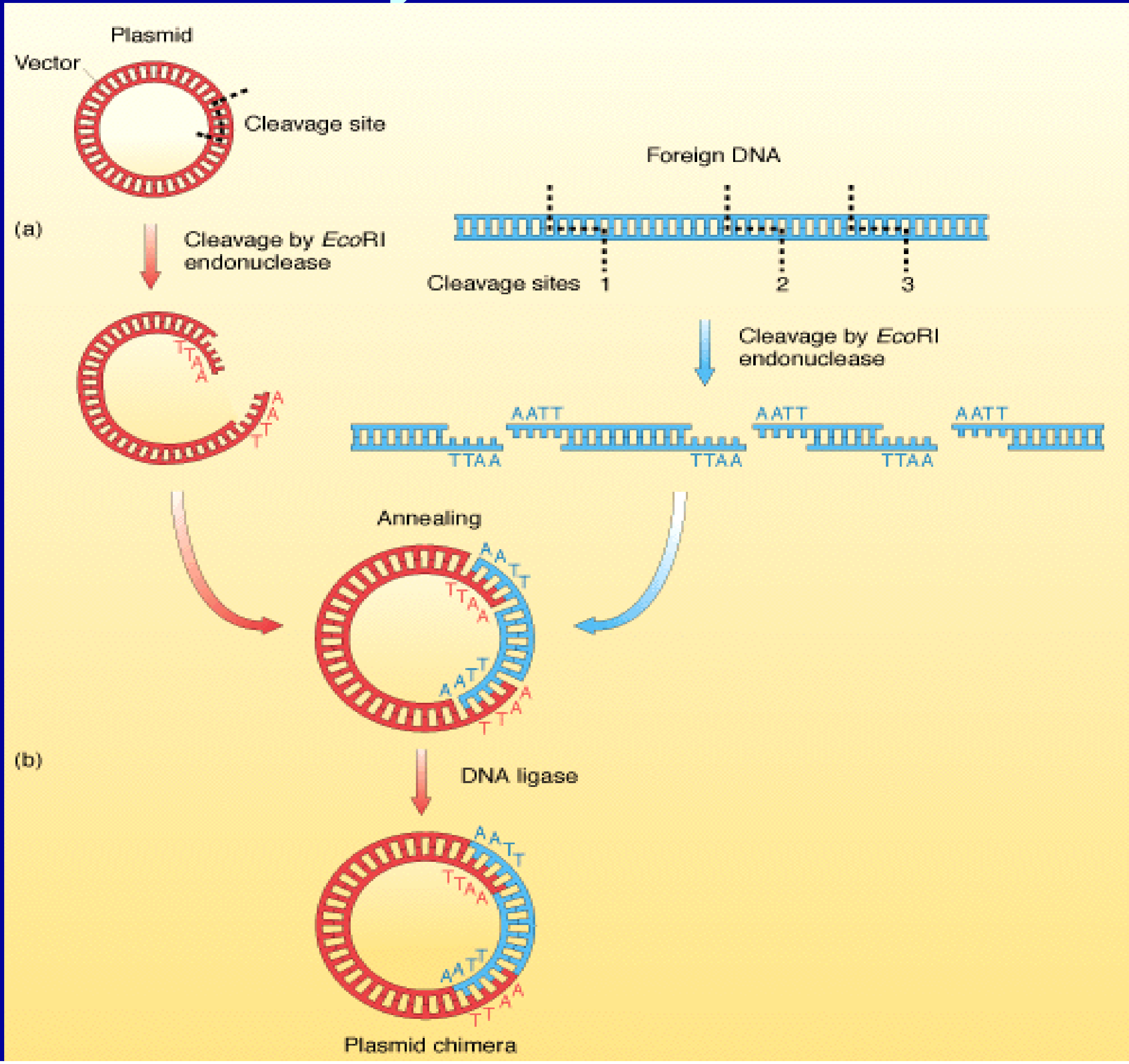


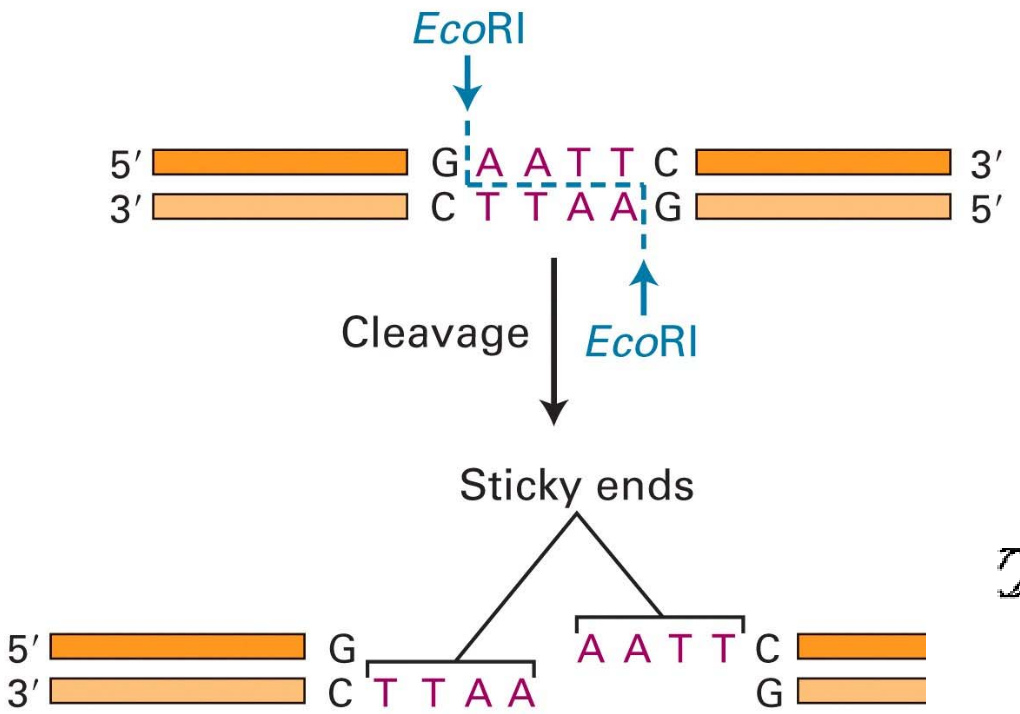
Restriction Enzymes, continued

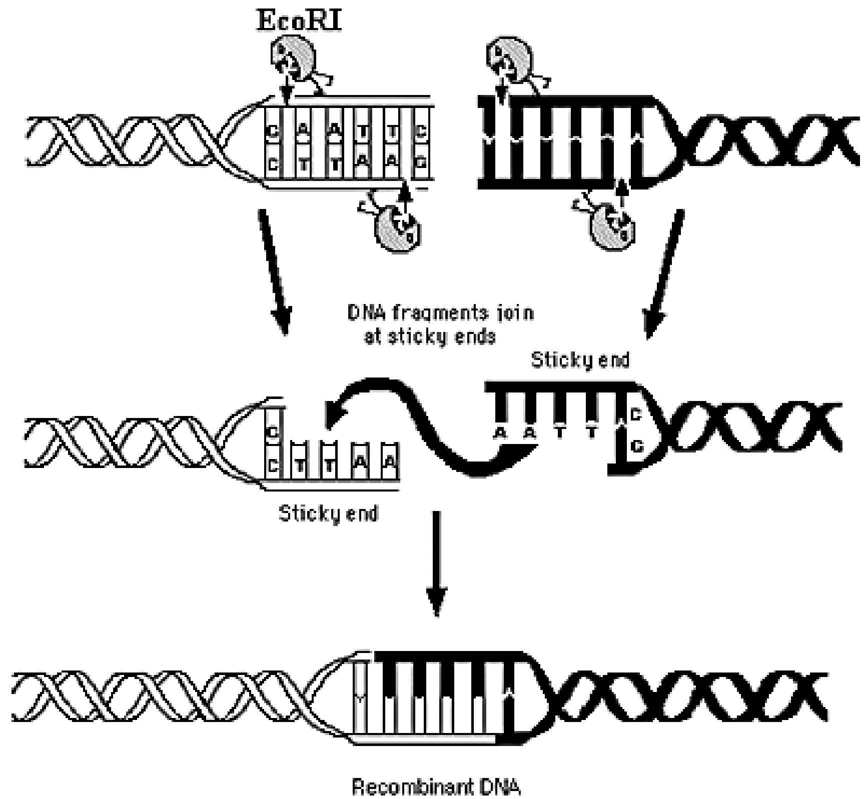


Restriction Enzymes, Cont.

- Any piece of DNA cut with a certain restriction enzyme will "stick" to any other piece cut with that same RE, even if they come from different sources.



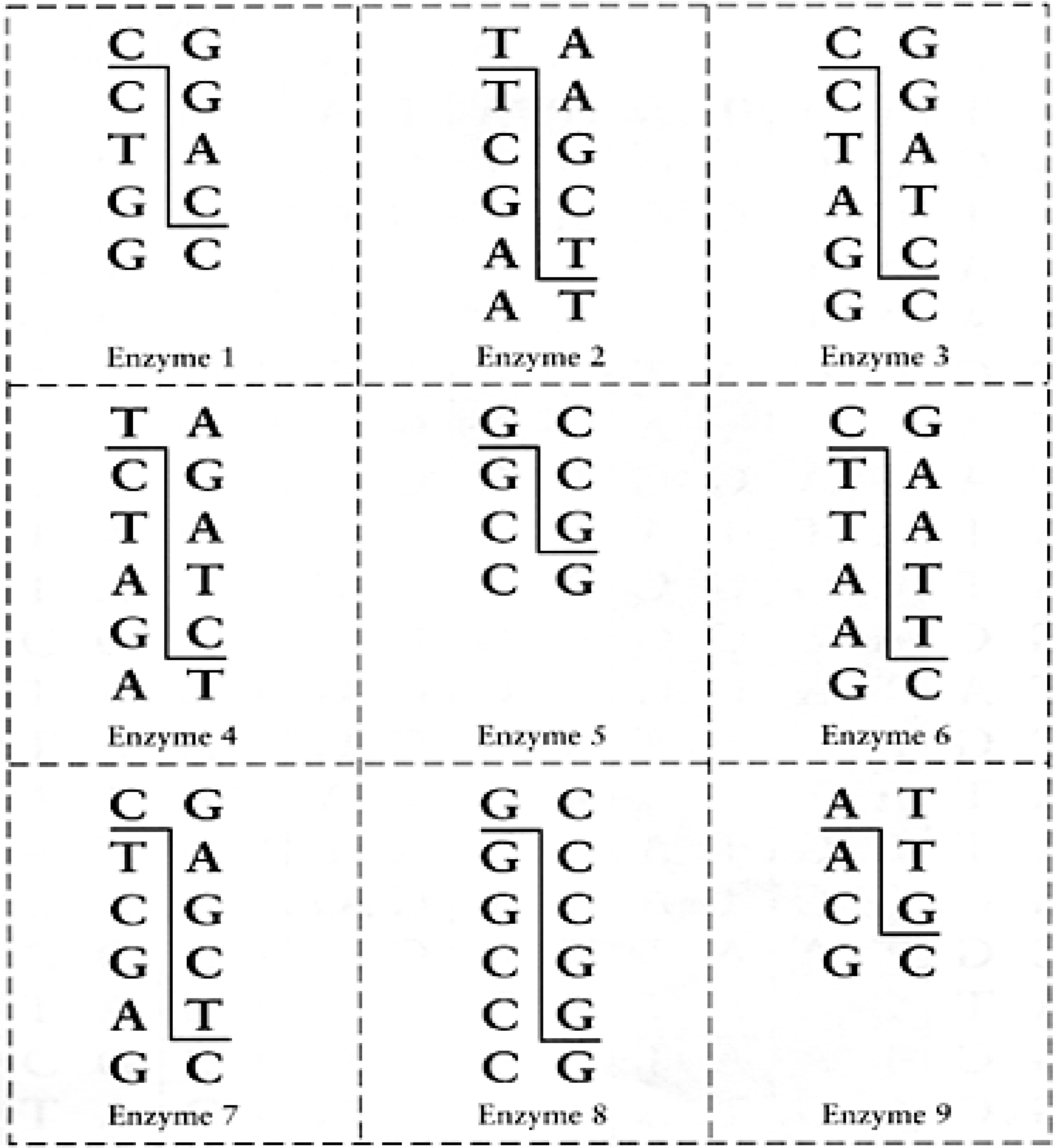




http://www.accessexcellence.org/AB/GG/restriction.html

Restriction Enzyme Action of EcoRI

What do you notice about these “restriction sites” (places where restriction enzymes cut)?



Cool, but what good are they?

Let’s find out…

Insulin for Diabetics: The Old Way

Insulin for Diabetics: The New Way

Step 1:

Isolate (find) the *human gene* responsible for producing insulin and decide where you want to put it.

In this case, we decide to put our human DNA into the *plasmid of E. coli*, a very common bacterium.

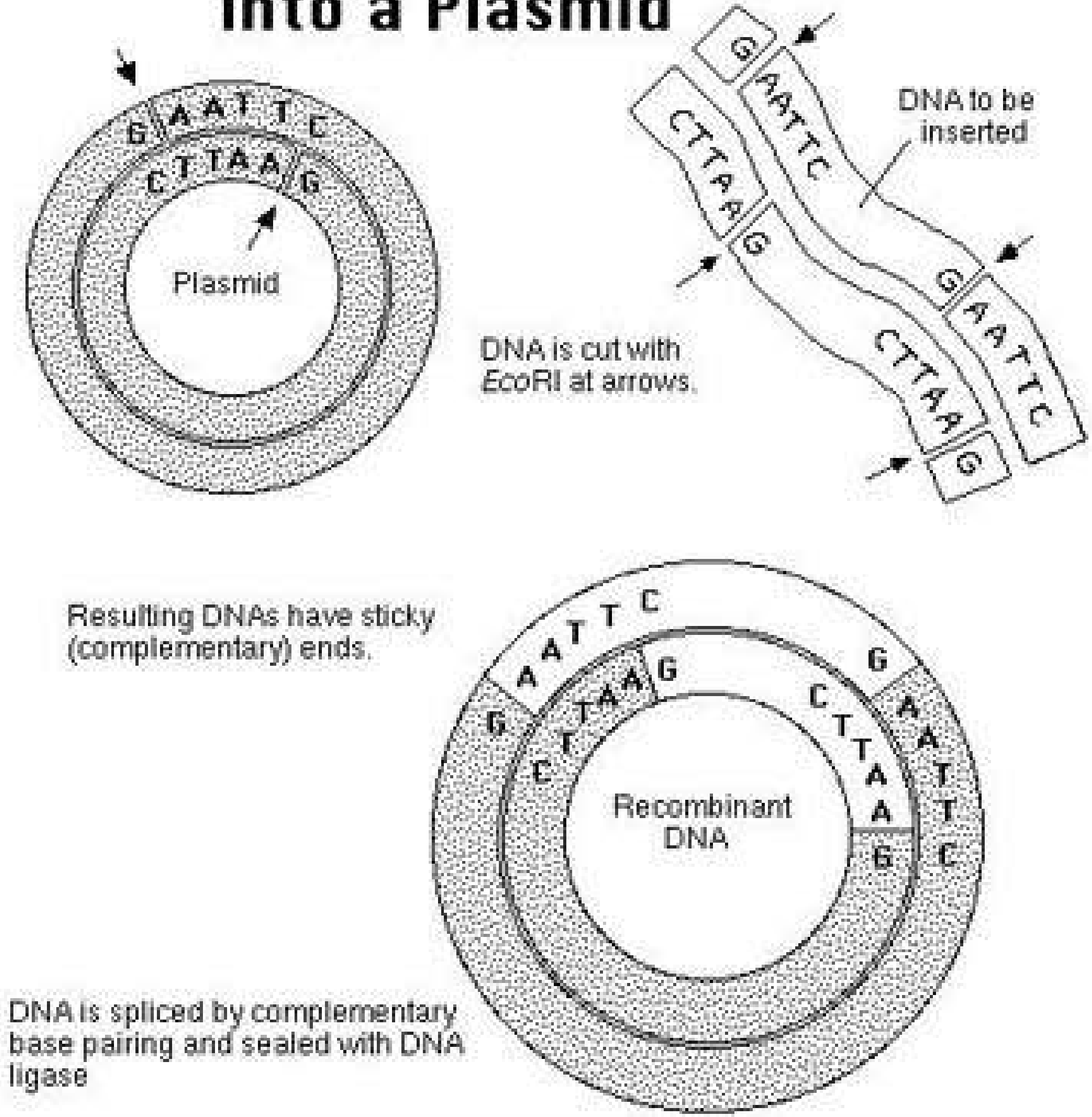
Step 2:

Get the bacterial (plasmid) DNA out of the E. coli. We do this by basically exploding them.

Step 3:

Cut your human DNA and bacterial DNA with the *same* restriction enzyme.

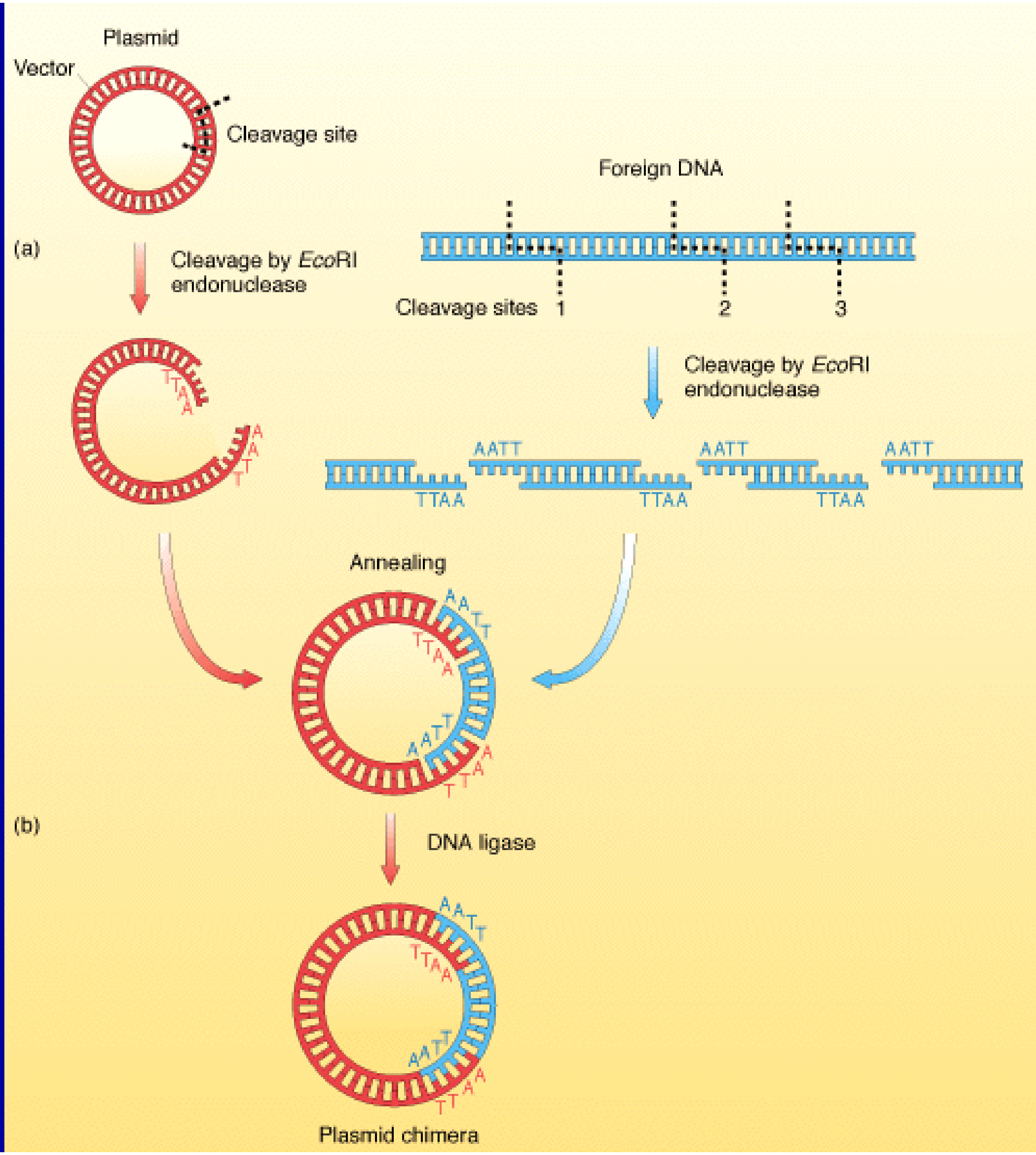
Inserting a DNA Sample into a Plasmid



Step 4:

Mix the cut human DNA, which contains the insulin gene, with the cut bacterial DNA.

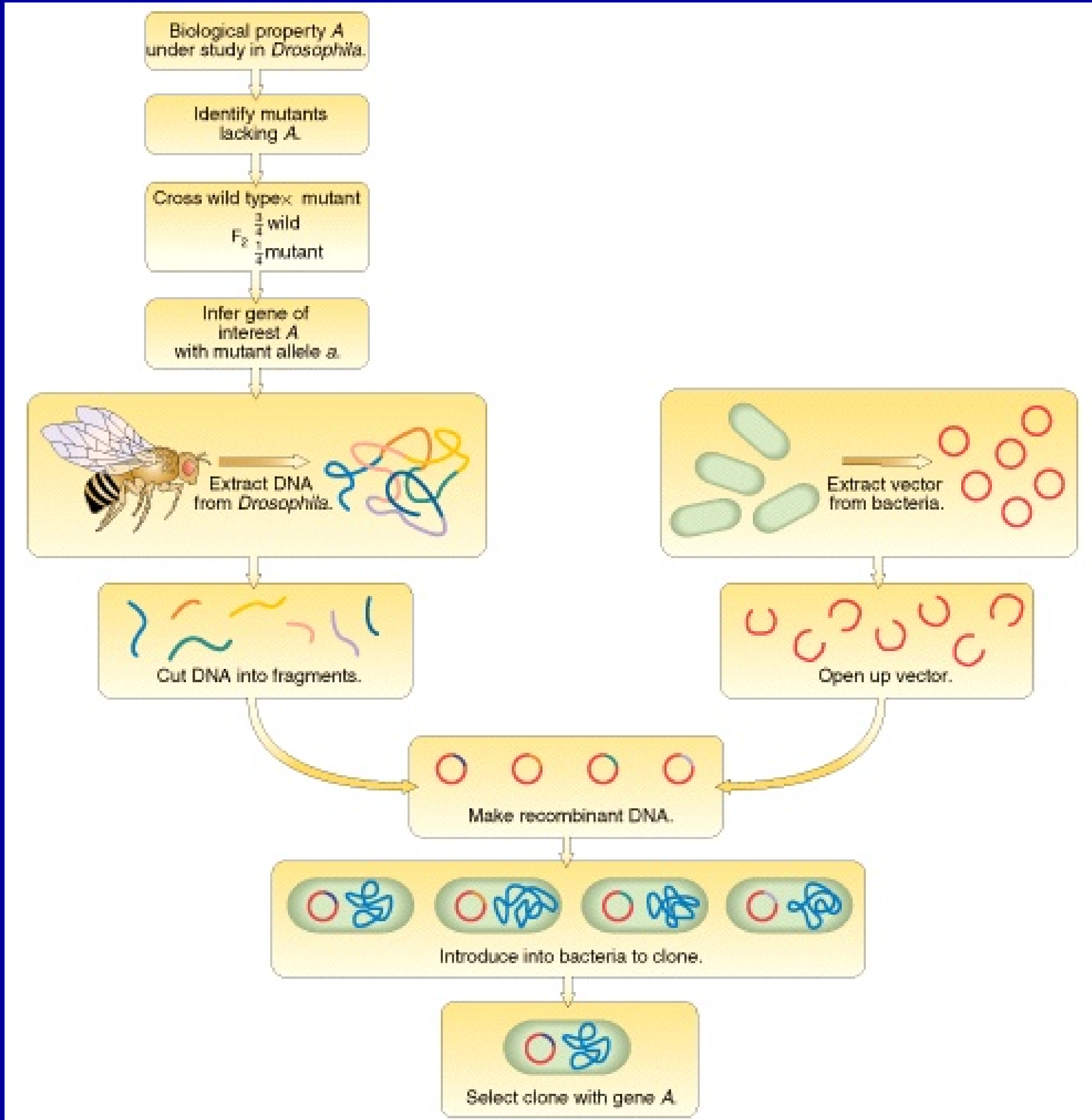
They'll *stick together* because they were cut with the same restriction enzyme.


Plasmid
Vector
Cleavage site
(a)
Cleavage by EcoRI endonuclease
Foreign DNA
Cleavage sites 1 2 3
Cleavage by EcoRI endonuclease
AATT
TTAA
Annealing
(b)
DNA ligase
Plasmid chimera

Step 5:

Get your new recombinant plasmid back into the bacteria.

This is easy because bacteria will take in DNA that's floating around near them. We call this *"transformation"*.

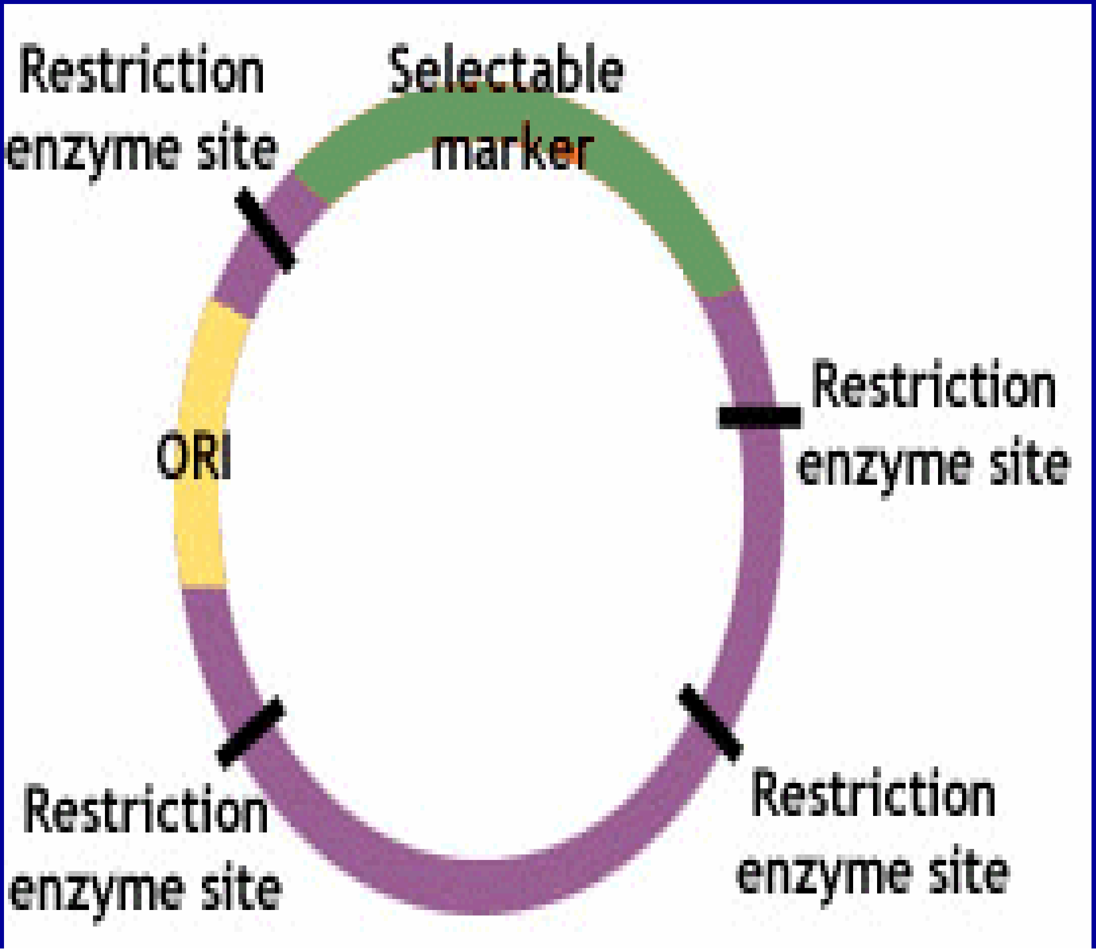


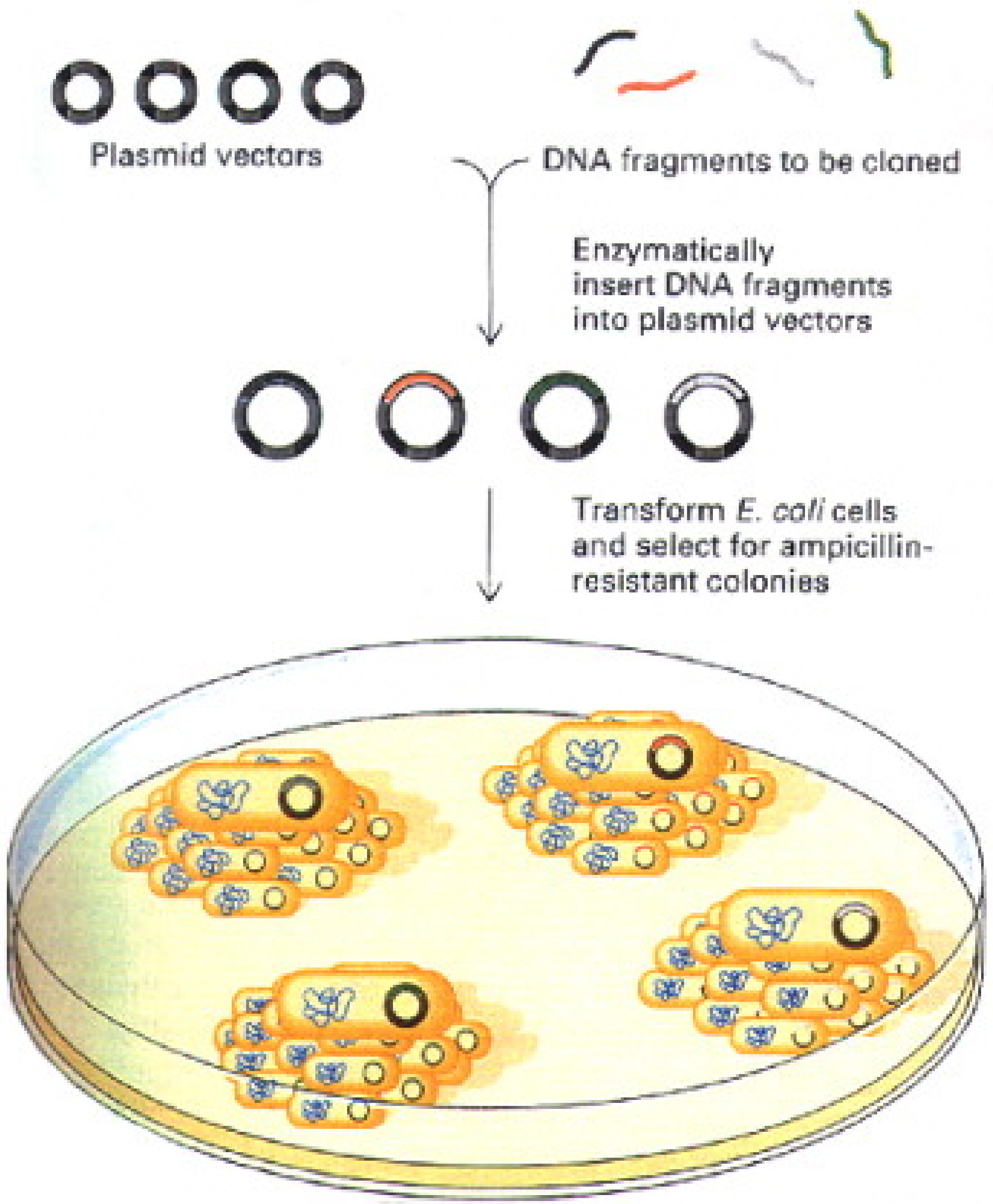
Biological property *A* under study in *Drosophila.*
Identify mutants lacking *A.*
Cross wild type × mutant
F_2 $\frac{3}{4}$ wild $\frac{1}{4}$ mutant
Infer gene of interest *A* with mutant allele *a.*
Extract DNA from *Drosophila.*
Extract vector from bacteria.
Cut DNA into fragments.
Open up vector.
Make recombinant DNA.
Introduce into bacteria to clone.
Select clone with gene *A.*

Step 6

- Find the bacteria that have taken up your recombinant plasmid amongst the riff-raff in the petri dish.

Remember how I said that many plasmids contain a gene that makes them resistant to antibiotics? How might you use that to find your special bacteria?




Plasmid vectors
DNA fragments to be cloned
Enzymatically
insert DNA fragments
into plasmid vectors
Transform *E. coli* cells
and select for ampicillin-
resistant colonies

Voila!!

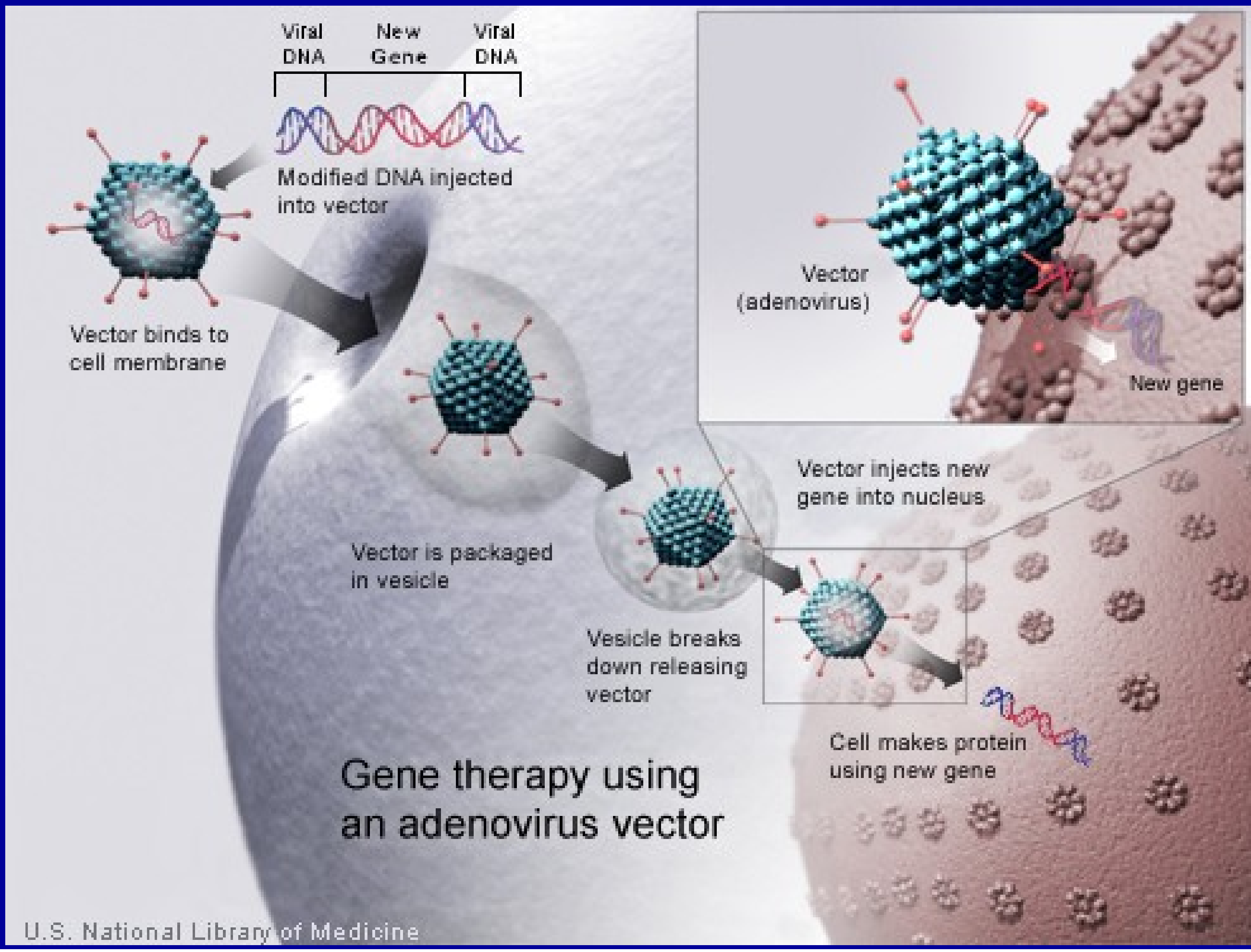
Now your E. coli will use its new DNA to make human insulin!

Because they reproduce so quickly, you'll soon have thousands, millions, or *billions* of human insulin making machines.

By filtering out the bacteria after they've made insulin, you've got clean human insulin that can be packaged and given to diabetic patients.

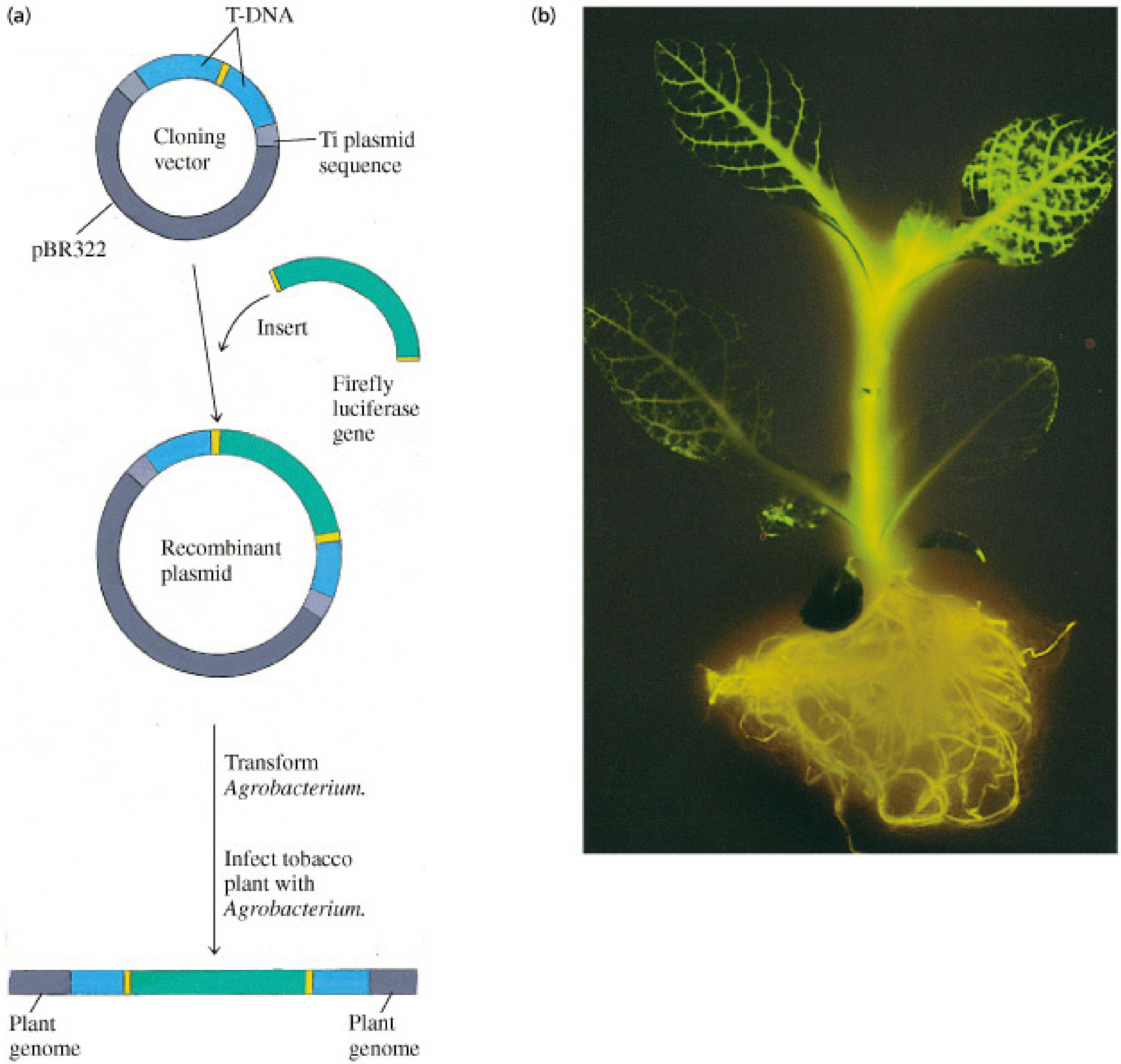
YAY!

Gene therapy: another way to use recombinant DNA



How do you think you could make a mouse glow in the dark?

Hint: Fireflies have a gene called "luciferase" that makes their little bums light up…



You can do it in plants too!

What genes do you think were introduced to this poor mouse's DNA?